ZEBRAFISH LARVAE AS A MULTI-SCALE MODEL TO STUDY THE NEUROTOXICITY OF METHYLMERCURY AND ITS PROTECTION BY SELENONEINE.

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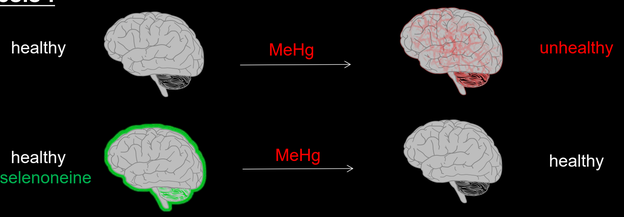
Introduction

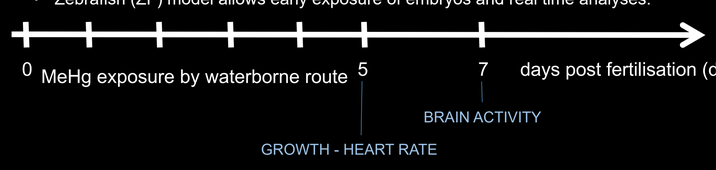
- Coastal populations worldwide are exposed to methylmercury (MeHg) through marine food consumption.
- Inuit are highly exposed to MeHg especially due to their consumption of beluga meat.
- Fetuses are most vulnerable to MeHg exposure, which affects brain neuronal development.
- Selenoneine is a putative anti-oxidative molecule found in Inuit's red blood cells that complexes with MeHg *(i)*.

- **Aim :** Investigate the protection afforded by selenoneine against MeHg neurodevelopmental toxicity.
- **Hypothesis :**



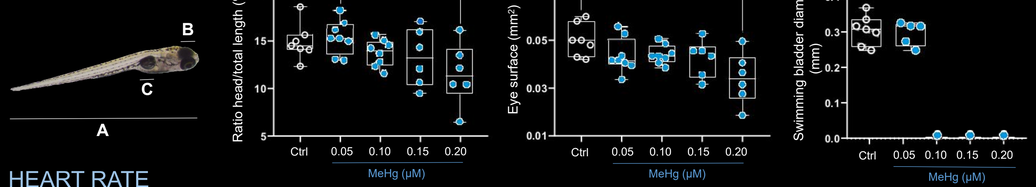
Experimental design

- Zebrafish (ZF) model allows early exposure of embryos and real time analyses.

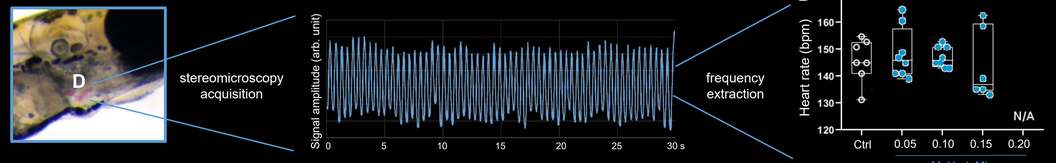


MeHg effects on ZF larvae development & physiology

GROWTH



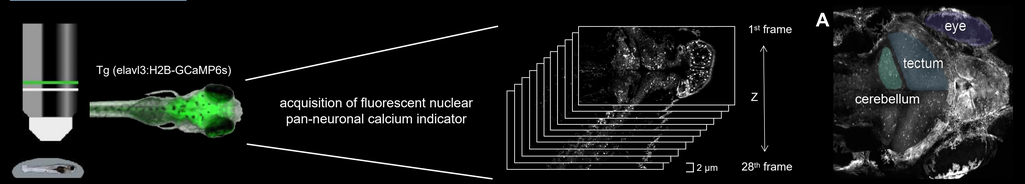
HEART RATE



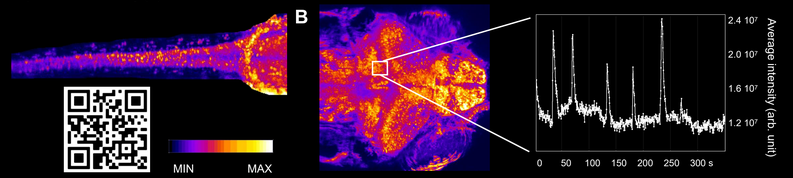
Single-blind measures on 5 dpf WT embryo. Morphometric (A, B, C) and heart beat measures (D). Box plots show means and extrema for each condition. Asterics denote significant differences (* : p<0.05, *** : p<0.005), assessed by a one-way ANOVA followed by Tukey post-hoc test.

Early results : MeHg exposure on ZF seems to impact the larval development in a dose-dependent manner. Effects on heart rate are quite variable and not significantly different.

BRAIN ACTIVITY



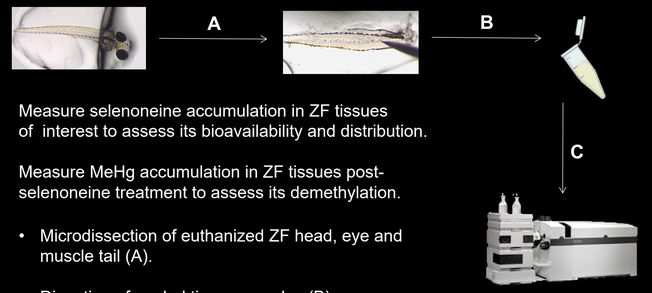
2-photon functional imaging on 7 dpf GCaMP larvae. Z-projection of anatomical stack with delineated regions of interest (A), and average intensity from 10 mn of neuronal spontaneous activity (B).



On-going work : investigation of MeHg impacts on ZF neurocircuits by assessing changes of spontaneous and evoked neuronal activity.

Next Steps

MeHg / selenoneine QUANTIFICATION



Measure selenoneine accumulation in ZF tissues of interest to assess its bioavailability and distribution.

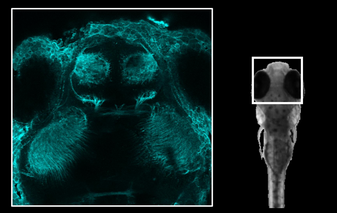
Measure MeHg accumulation in ZF tissues post-selenoneine treatment to assess its demethylation.

- Microdissection of euthanized ZF head, eye and muscle tail (A).
- Digestion of pooled tissue samples (B).
- MeHg and selenoneine quantified by gas (MeHg) or liquid (selenoneine) chromatography coupled with inductively coupled plasma - mass spectrometry (C).

IMMUNOSTAINING

Whole-mount immunofluorescence to monitor :

- apoptosis events (anti-active caspase 3 antibody), and
- MeHg distribution (anti-MeHg antibody) in exposed-ZF embryo.



Axonal tracts labeled by anti-acetylated tubulin antibody in 5 dpf WT embryo, imaged on confocal microscope.

References & Acknowledgments

(i) Achouba et al., 2019. Selenoneine is a major selenium species in beluga skin and red blood cells of Inuit from Nunavik. Chemosphere.

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